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Effect of Nisin in Combination with EDTA, Sodium Lactate, and Potassium Sorbate for Reducing *Salmonella* on Whole and Fresh-Cut Cantaloupe†

DIKE O. UKUKU* AND WILLIAM F. FETT

Food Safety Intervention Technologies Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

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ABSTRACT

Nisin (50 μg/ml), EDTA (0.02 M, disodium salt), sodium lactate (NaL, 2%), and potassium sorbate (KS, 0.02%) were tested individually and in various combinations as sanitizer treatments for reducing *Salmonella* on whole and fresh-cut cantaloupe. Whole cantaloupe and fresh-cut pieces were inoculated with a five-strain cocktail of *Salmonella* to give 4.76 ± 0.23 log CFU/cm^2 and 3.42 ± 0.13 log CFU/g, respectively. Inoculated whole melons and fresh-cut pieces were stored at 5°C for 7 days. Washing treatments were applied to inoculated whole melons at days 0, 3, and 7 of storage, and surviving bacterial populations were determined. The effect of the washing treatments on transfer of *Salmonella* to fresh-cut pieces prepared immediately after treatment was also determined. Directly inoculated fresh-cut pieces were treated at day 0, and surviving bacteria were enumerated at days 0, 3, and 7 of storage. The combination treatments of nisin-EDTA, nisin-NaL, nisin-KS, NaL-KS, and nisin-NaL-KS all resulted in reductions of approximately 3 log CFU/cm^2 at day 0 for whole melons. When tested alone, all compounds, along with water washes, were ineffective. After 3 and 7 days of storage, the five combination washing treatments were less effective, resulting in reductions of approximately 2 log CFU/cm^2. None of the combination treatments completely eliminated transfer of pathogen survivors to fresh-cut pieces. The combination treatments nisin-NaL, nisin-KS, NaL-KS, and nisin-NaL-KS, but not nisin-EDTA, gave significant ($P < 0.05$) reductions of *Salmonella* directly inoculated onto fresh-cut pieces. Washing with nisin-NaL-KS was significantly ($P < 0.05$) more effective than the other three combination treatments, resulting in a reduction of 1.4 CFU/g. Inhibition by the four effective treatments carried over from day 0 through day 7 of storage, with no increase in the population of *Salmonella* on the stored fresh-cut pieces. Sensory evaluations indicated that treatment of fresh-cut pieces with nisin-NaL and NaL-KS, but not nisin-KS or nisin-NaL-KS, were acceptable in terms of appearance, odor, and overall acceptability. After the required regulatory approval, treatment of whole cantaloupe with nisin in combination with EDTA, NaL, KS, or NaL and KS and of fresh-cut pieces with nisin-NaL or NaL-KS could help ensure the microbiological safety of fresh-cut cantaloupe.

During growth in the field, cantaloupe surfaces, like most vegetables, are frequently in contact with the soil *(4)*, and their surfaces are not free from natural contaminants. The surface of cantaloupe muskmelon is covered with a meshwork of raised tissue commonly referred to as a ''net'' *(37)*. Several cases of foodborne illness from consumption of melons contaminated on their surfaces with the bacterial human pathogen *Salmonella* have been reported *(8, 13)*. Therefore, the microbiological safety of fresh-cut melon and other produce available in salad bar operations and supermarkets is a concern *(14, 36)*. The level of sanitation and the microbiological load are of primary importance to the quality, shelf stability, and safety of fresh produce *(6, 19, 20)*.

Physical and chemical treatments are used in food processing to eliminate or at least reduce the presence of pathogenic and spoilage microorganisms *(23, 31, 33, 38)*. Washing is one of the first processing operations to which a fruit or vegetable is subjected. Recently, we reported that treatment of whole and fresh-cut cantaloupe and honeydew melon with a combination of nisin-EDTA significantly reduced the natural microflora and extended the shelf life *(31)*.

Nisin is a pentacyclic heterodetic subtype A lantibiotic peptide synthesized by *Lactococcus lactis* subsp. *lactis (12, 15, 16, 26)*. It is an effective inhibitor of gram-positive bacteria *(3, 9, 13, 23, 35)* and bacterial spores *(23)*. In the United States, nisin has received generally recognized as safe (GRAS) status and is approved for use in some processed cheese spreads to prevent the outgrowth of clostridial spores and toxin production *(3)*. In several cases, nisin used in combination with a chelating agent has been reported to exhibit a bactericidal effect toward both gram-positive and gram-negative bacteria *(5, 10, 28–30)*. In this study, the efficacy of nisin treatments in combination with EDTA, sodium lactate, or potassium sorbate in reducing the population of *Salmonella* inoculated on whole cantaloupe and fresh-cut pieces and reducing transfer from the inoculated whole melon surface to fresh-cut pieces was investigated. Also, the appearance, odor, and overall acceptability of fresh-cut pieces dipped in various combinations of the

* Author for correspondence. Tel: 215-233-6427; Fax: 215-233-6406; E-mail: dukuku@errc.ars.usda.gov.

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antimicrobial compounds were monitored during storage at 5°C for up to 7 days.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation. Bacterial strains used in this study were *Salmonella* Stanley H0558, *Salmonella* Newport H1275, *Salmonella* Anatum F4317, *Salmonella* Infantis F4319 (all associated with alfalfa sprout–related outbreaks, obtained from Dr. Patricia Griffin, Centers for Disease Control), and *Salmonella* Poona RM2350 (associated with a cantaloupe-related outbreak, obtained from Dr. Robert Mandrell, Agricultural Research Service, U.S. Department of Agriculture). Bacteria were maintained on brain heart infusion agar (BBL/Difco, Becton Dickinson, Sparks, Md.) slants held at 4°C. Prior to use, the cultures were subjected to two successive transfers by loop inocula to 5 ml of brain heart infusion broth (BBL/Difco). A final transfer of 0.2 ml was made into 20 ml of brain heart infusion broth with incubation at 36°C for 18 h under static conditions. Bacterial cells were harvested by centrifugation (10,000 $\times$ *g*, 10 min) at 4°C, and the cell pellets were washed in salt-peptone (0.85% NaCl, 0.05% Bacto Peptone [BBL/Difco]). An inoculum cocktail containing all five *Salmonella* serovars at 2.13 $\times$ 10^8 CFU/ml of *Salmonella* Poona RM2350, 2.10 to 2.63 $\times$ 10^8 CFU/ml of *Salmonella* Stanley H0558, 1.87 to 2.20 $\times$ 10^8 CFU/ml of *Salmonella* Newport H1275, 1.92 to 2.34 $\times$ 10^8 CFU/ml of *Salmonella* Anatum F4317, and 1.68 to 1.97 $\times$ 10^8 CFU/ml of *Salmonella* Infantis F4319 was prepared in 3 liters of 0.1% (wt/vol) peptone water and used to inoculate whole melons. A second inoculum cocktail containing all five *Salmonella* serovars at 1.06 to 1.24 $\times$ 10^6 CFU/ml of *Salmonella* Poona RM2350, 9.89 $\times$ 10^5 to 1.13 $\times$ 10^6 CFU/ml of *Salmonella* Stanley H0558, 1.04 to 1.27 $\times$ 10^6 CFU/ml of *Salmonella* Newport H1275, 1.10 to 1.31 $\times$ 10^6 CFU/ml of *Salmonella* Anatum F4317, and 8.91 $\times$ 10^5 to 1.07 $\times$ 10^6 CFU/ml of *Salmonella* Infantis F4319 was prepared in 3 liters of 0.1% (wt/vol) peptone water and was used to inoculate the fresh-cut melon.

In vitro antimicrobial assays. Cocktails (10^8 CFU/ml) of the *Salmonella* serovars (1 ml) prepared as described above were added to 20 ml of tryptic soy broth (TSB, pH 4 to 6.8; BBL/Difco) containing each of the antimicrobial solutions (see ''Inoculation of whole melon and fresh-cut pieces'') to be tested. The initial population of *Salmonella* in the test medium was 3.45 $\pm$ 0.13 log CFU/ml. For initial tests, the pH of the medium was adjusted to the desired value with 2 N HCl (Mallinckrodt Baker Inc., Paria, Ky.) after addition of the antimicrobial compounds and before inoculation. A pH of 6.8 was adopted for the rest of the study because cantaloupe fresh-cut pieces have a similar pH value. The inoculated test media were incubated at 5, 25, and 36°C (static) for up to 24 h, and surviving cells were determined at 0, 3, 6, 9, 15, and 24 h by pour plating on Trypticase soy agar (BBL/Difco) with incubation at 37°C for 24 h.

Preparation of sanitizer solutions. A stock solution of nisin (10^6 IU, Sigma, St. Louis, Mo.) was prepared at a concentration of 2500 μg/ml in 0.02 N HCl. Sodium lactate (NaL; 60% w/v Sigma) was prepared at a concentration of 20% in deionized distilled water (ddH_2O). Potassium sorbate (KS; Fisher Scientific Co., Pittsburgh, Pa.) was prepared at a concentration of 1% in ddH_2O. These stock solutions were filter sterilized (0.22 μm, Millipore, Bedford, Mass.). A stock solution of 2 M disodium EDTA (Fisher Scientific) was prepared in ddH_2O, autoclaved at 121°C for 15 min, and stored at room temperature until used. Test solutions were prepared by dilution with sterile ddH_2O as required. Final concentrations of the chemicals used alone or in combination were 0.02 M (EDTA), 50 μg/ml (nisin), 2% (NaL), and 0.02% (KS).

Inoculation of whole melon and fresh-cut pieces. Unwaxed whole cantaloupes (1,461.5 to 1,548.1 g western shippers) purchased from a local distributor were allowed to come to room temperature (~20°C) overnight before being inoculated. Cantaloupes were submerged in 3 liters of bacterial inoculum cocktail (10^8 CFU/ml) prepared as described above and rotated by stirring with a glove-covered hand for 10 min to ensure even inoculation. The inoculated cantaloupes were air dried for 1 h in a biosafety cabinet and stored at 5°C for up to 7 days before washing with antimicrobial agents listed above (''In vitro antimicrobial assays'') at 0, 3, or 7 days postinoculation and sampling (see ''Microbiological analyses'').

To prepare fresh-cut pieces, inoculated or uninoculated whole cantaloupes were cut into four sections with a sterile knife, and the rinds were carefully removed. The interior flesh was cut into ~3-cm cubes, and pieces were investigated for presence of *Salmonella* transferred from inoculated melons. Also, fresh-cut pieces prepared from uninoculated melons were inoculated by dipping in a cocktail of the five *Salmonella* serovars (10^6 CFU/ml) prepared as described above for 2 min to achieve an initial population of 10^3 CFU/g. Inoculated fresh-cut pieces were placed inside a basket and left inside a biosafety cabinet for 3 h to dry before washing with antimicrobial solutions as described before.

Washing treatments. Antimicrobial solutions of nisin, EDTA, NaL, KS, nisin-EDTA, nisin-EDTA-NaL, nisin-EDTA-KS, or nisin-EDTA-NaL-KS prepared as stated above were tested as sanitizers for inoculated whole cantaloupes. Washing treatments for inoculated whole melons were performed by totally submerging the melons in 3 liters of sterile tap water or in a sanitizer solution and manually rotating for 5 min to assure complete coverage and contact of surfaces with the wash solution. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h. Inoculated fresh-cut pieces were washed for 1 min with sterile tap water, nisin-EDTA, nisin-EDTA-NaL, nisin-EDTA-KS, and nisin-EDTA-NaL-KS before placing inside a Stomacher (Dynatech Laboratories, Alexandria, Va.) bag (100 g per bag). Bags containing the pieces were stored at 5°C for 7 days.

Microbiological analyses. A sterilized stainless steel cork-borer was used to cut through whole melon surfaces at random locations to produce rind plugs of 22 mm in diameter with a rind surface area (πr^2) of 3.80 cm^2. Flesh adhering to the rind plugs was trimmed off with a sterilized stainless steel knife. Sixty melon rind plugs per whole melon weighing approximately 20 g were blended (Waring commercial blender, speed set at level 5, Dynamic Corp., New Hartford, Conn.) for 1 min with 80 ml of sterile 0.1% peptone water. Decimal dilutions of the sample were made with 0.1% peptone water, and aliquots (0.1 ml) were plated in duplicate on bismuth sulfite agar (BSA; BBL/Difco).

For fresh-cut pieces, 200 ml of 0.1% peptone water was added to the Stomacher bags containing fresh-cut pieces, and the bag contents were pummeled for 30 s in a Stomacher model 400 (Dynatech) at medium speed. Undiluted samples or samples from decimal serial dilutions prepared in 0.1% peptone water were plated in duplicate on BSA media (0.1 ml per plate) with incubation at 35°C for 48 h. For preenrichment, the interior flesh was cut into ~3-cm cubes, and 100 g of the cubes was placed in a Stomacher bag along with 200 ml of Difco nutrient broth and pummeled for 30 s in a Stomacher model 400 at medium speed with incubation at 35°C for 18 to 22 h. For selective enrichment, a 1-ml aliquot

TABLE 1. *Rating scale for appearance, odor, and overall acceptability for fresh-cut melons*

Measurement	Score[a]	Quality description
Appearance	1	Dislike extremely; very poor, not usable
	3	Dislike moderately; poor, excessive defects, limited salability
	5	Neither like nor dislike; borderline, fair, slightly to moderately objectionable defects, lower limit of appeal
	7	Like moderately; good, minor defects, not objectionable
	10	Like extremely; excellent, essentially free from defects, fresh-like and typical
Odor	1	Dislike extremely
	3	Unacceptable; poor, stale, musty, and moldy
	5	Fairly acceptable
	7	Good; not objectionable, acceptable
	10	Excellent; typical, very much acceptable
Overall acceptability	1	Dislike; extremely poor
	3	Dislike; moderately poor
	5	Neither like nor dislike; fair, may buy from the store
	7	Very good; will definitely buy
	10	Extremely good; most definitely buy

[a] Panelists were asked to use intermediate scores where they deemed appropriate.

of the preenriched sample was added to 9 ml of tetrathionate broth (Difco) and incubated at 35°C for 24 h. Enrichment cultures were plated on BSA agar (0.1 ml per plate) in duplicate and incubated at 35°C for 24 h. For comparison, a pure culture of *Salmonella* Poona was plated on BSA, incubated as above, and run parallel with the samples. For comparison, a pure culture of *Salmonella* Poona was plated on BSA, incubated as above, and run parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the U.S. Food and Drug Administration *Bacteriological Analytical Manual* following conventional biochemical methods *(2).*

Quality evaluation. Panelist selection and training and quality evaluation were undertaken as described by O'Connor-Shaw et al. *(22),* which was a slight modification of a procedure described by Kader et al. *(17).* Pieces of fresh-cut melon were scored for appearance, odor, and overall acceptability with the use of a predetermined list of descriptors (Table 1). A panel of five judges was used to evaluate the quality of fresh-cut melon during refrigerated storage (5°C) at days 0, 3, 6, 9, 12, 15, 18, and 21. On each day of testing, panelists were presented with freshly cut untreated melon pieces as a reference.

Data analysis. All experiments were replicated three times. Data from each treatment were subjected to Statistical Analysis Systems software (SAS Institute, Cary, N.C.) for analysis of variance (ANOVA) and the Bonferroni least significant difference method *(21)* to determine significant differences between treatments and storage temperatures for up to 21 days.

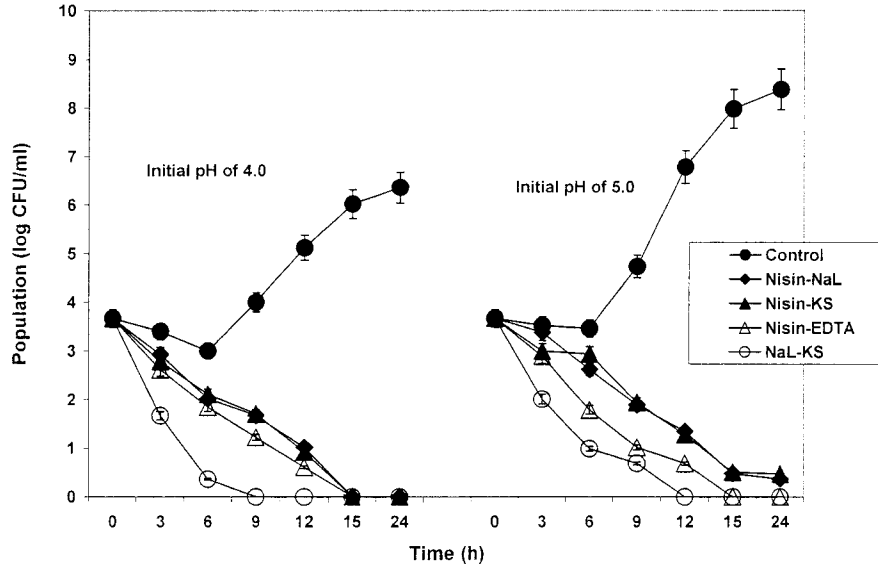
RESULTS

In vitro antibacterial activity. The addition of nisin, EDTA, and NaL alone to TSB (pH 4 to 6.8) inoculated with a five-strain cocktail of *Salmonella* had no effect on the pathogen growth. Addition of KS alone resulted in a slight 0.4-log reduction of the maximum pathogen population reached (data not shown). The effect of nisin-EDTA, nisin-NaL, nisin-KS, and nisin-NaL-KS in reducing the population of a five-strain cocktail of *Salmonella* in a nutrient-rich environment (TSB) at pH 4 and 5 is shown in Figure 1. The *Salmonella* population increased to approximately 10^7 and 10^9 CFU/ml in TSB at pH 4 and 5, respectively, without added antimicrobials during incubation for 24 h at 36°C. The antimicrobial effect of all combinations of the compounds added to TBS was bactericidal, with bacterial populations below the level of detection (<2 CFU/ml) in TSB adjusted to an initial pH of 4.0 by 15 h at 36°C. Similar bactericidal activity was noted in TSB at pH 5.0, but low levels of survivors were still detectable in TSB amended with nisin-KS and nisin-NaL at 24 h. The combination of NaL-KS was the most effective antimicrobial agent tested at both pHs, leading to the most rapid decline in viable *Salmonella* in the broth.

When the broth pH was increased to 6.8 with incubation at 25 or 36°C for 24 h, all combinations of antimicrobials were still bactericidal except for nisin-EDTA, which was now bacteriostatic (Fig. 2). The broth pH (6.8) matched the pH measured for cantaloupe flesh homogenates in ddH_2O. The bactericidal activity of the NaL-KS combination was slightly enhanced at 36°C over the activity at 25°C

Effect of washing treatments on *Salmonella* inoculated onto whole melons. In preliminary experiments, washing inoculated whole melons at day 0 with water, NaL, KS, EDTA, and nisin, when tested individually, did not cause significant ($P > 0.05$) reductions in *Salmonella* populations (data not shown). Thus, combinations of nisin, EDTA, NaL, and KS were tested as sanitizer treatments (Table 2). All the combination treatments significantly ($P < 0.05$) reduced *Salmonella* populations on whole-melon surfaces. The log reductions obtained before storage of the inoculated melons were approximately 3 log units. Efficacy of all treatments was diminished after storage of inoculated melons for 3 or 7 days at 5°C before treatment (Table 2).

Effect of sanitizing treatments on populations of *Salmonella* on fresh-cut pieces. Populations of *Salmonella* transferred from unwashed (control) or washed, inoculated



–FIGURE 1. *Survival of a five-strain cocktail of* Salmonella *tryptic soy broth at pH 4 and 5 amended with nisin (50 μg/ml)-NaL (2%), nisin (50 μg/ml)-KS (0.02%), nisin (50 μg/ml)-EDTA (0.02 M), and NaL (2%)-KS (0.02%) and incubated at 36°C.*

whole cantaloupes to fresh-cut pieces during cutting are shown in Table 3. Fresh-cut pieces were prepared after storage of inoculated melons for up to 7 days. Populations of *Salmonella* determined in fresh-cut pieces prepared from untreated inoculated whole melons stored at 5°C for 0, 3, and 7 days showed a gradual increase, but the populations were not significantly ($P > 0.05$) different from each other. At days 0 and 3, survivors were not detected by direct plating but were detected by enrichment (Table 3). At day 7, *Salmonella* was detected in all treated fresh-cut pieces by direct plating and enrichment except those pieces prepared from whole melons washed in nisin-NaL-KS (enrichments only).

Fresh-cut pieces directly inoculated with *Salmonella* were examined for survivors after sanitizing treatments and followed by storage at 5°C for up to 7 days (Table 4). The initial population of *Salmonella* on inoculated fresh-cut pieces was 3.42 log CFU/g. Washing with water or nisin-EDTA was ineffective. Significant log reductions (0.6 to 1.0 log CFU/g, $P < 0.05$) were obtained with nisin-NaL, nisin-KS, and nisin-Nal-KS, and the three treatments were not significantly different from each other. The most effective treatment was the combination of nisin-NaL-KS, resulting in a 1.4 log CFU/g reduction. Unlike the results for *Salmonella* inoculated on whole melons, populations of *Salmonella* inoculated on fresh-cut pieces prepared from untreated and water-washed melons slightly increased throughout storage. Populations in fresh-cut pieces treated with the combination of antimicrobial compounds remained steady throughout storage at 5°C.

To investigate whether treatments with nisin-NaL and NaL-KS can control growth of *Salmonella* on inoculated fresh-cut cantaloupe pieces during storage at an abusive temperature (10°C), fresh-cut pieces inoculated to a final concentration of 3.7 log CFU/g were treated with the antimicrobial solutions and then stored at 5 and 10°C for 21 days. In this particular set of experiments, *Salmonella* populations in nontreated fresh-cut pieces did not increase during storage at 5°C but did increased to 7 log CFU/g during storage at 10°C (Fig. 3). Both combination treatments completely inhibited growth of *Salmonella* throughout the 21-day storage period at both storage temperatures, and populations gradually declined.

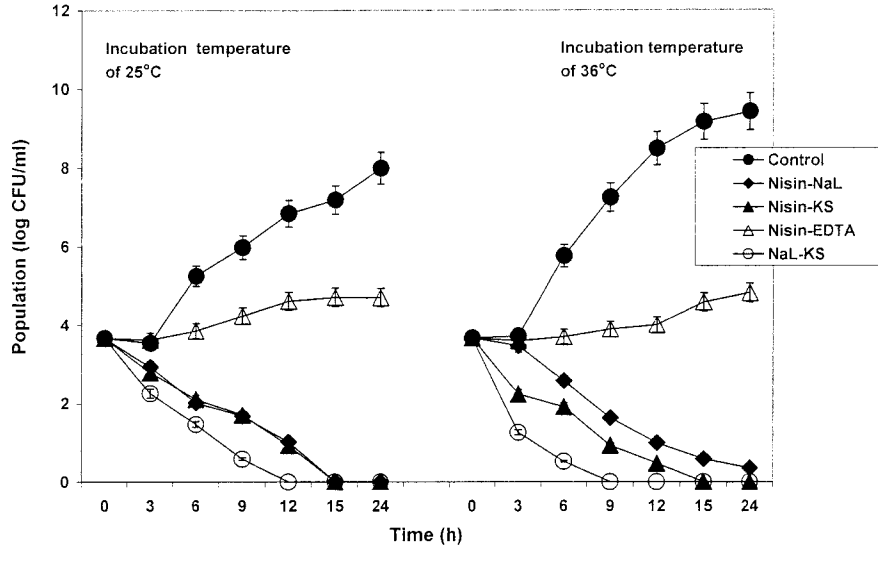


FIGURE 2. *Survival of a five-strain cocktail of* Salmonella *tryptic soy broth at pH 6.8 amended with nisin (50 μg/ml)-NaL (2%), nisin (50 μg/ml)-KS (0.02%), nisin (50 μg/ml)-EDTA (0.02 M), and NaL (2%)-KS (0.02%) and incubated at 25 and 36°C.*

TABLE 2. *Survival of* Salmonella *on whole cantaloupe after storage (5°C) for up to 7 days and washing treatments*

	Population[b]					
	Day 0		Day 3		Day 7	
Treatments[a]	Log CFU/cm^2	Log reduction	Log CFU/cm^2	Log reduction	Log CFU/cm^2	Log reduction
Control	4.76 ± 0.23 A		4.67 ± 0.14 A		4.52 ± 0.13 A	
Water	4.54 ± 0.20 A	0.22	4.44 ± 0.12 A	0.23	4.36 ± 0.15 A	0.16
Nisin-EDTA	1.66 ± 0.13 B	3.10	2.59 ± 0.15 B	2.08	2.66 ± 0.14 B	1.86
Nisin-NaL	1.50 ± 0.10 B	3.26	2.52 ± 0.11 B	2.15	2.46 ± 0.13 B	2.06
Nisin-KS	1.40 ± 0.14 B	3.36	2.40 ± 0.13 B	2.27	2.36 ± 0.12 B	2.16
NaL-KS	1.70 ± 0.13 B	3.06	2.66 ± 0.10 B	2.01	2.70 ± 0.11 B	1.82
Nisin-NaL-KS	1.32 ± 0.12 B	3.44	2.22 ± 0.13 B	2.45	2.26 ± 0.10 B	2.26

[a] Concentrations of antimicrobial agents were 50 μg/ml (nisin), 0.02 M (EDTA), 2% (NaL), and 0.02% (KS).

[b] Values are means ± standard deviations of three trial experiments with duplicate determinations per trial. Means in the same column for each day of storage not followed by the same letter are significantly ($P < 0.05$) different.

Effect of treatments on the quality of fresh-cut melon. Sensory evaluation ratings for changes in appearance and odor for fresh-cut melons directly inoculated with *Salmonella* and then treated with antimicrobial compounds with storage at 5°C for 7 days is shown in Table 5. The minimum acceptable limit for this study was set at a rating of 5 and above for each of the three categories. There were no differences in ratings between the untreated control (control 1) and the control inoculated with *Salmonella* at 3.65 log CFU/g without treatment (control 2) prepared on the day of evaluation. Acceptable ratings for all three factors were obtained for EDTA, NaL, nisin-NaL, and NaL-KS. Fresh-cut pieces treated with nisin-NaL-KS were not acceptable for any factor.

DISCUSSION

Because of the numerous foodborne outbreaks of salmonellosis associated with contaminated fresh-cut cantaloupe and because the pathogen most likely originates from the contaminated rinds of whole melons, the application of effective antibacterial interventions to the surface of the whole melon as well as the surface of fresh-cut fruits is desirable. In this study, we tested several antimicrobial compounds alone or in combination for this purpose including nisin, lactic acid, sorbic acid, and EDTA.

All compounds tested in this study are GRAS and are used in foods as antimicrobials *(11)*. Lactic acid (pK_a = 3.08) and its sodium and potassium salts are added to foods as acidulants as well as emulsifiers, humectants, and flavor enhancers *(24)*. The addition of sodium and potassium lactate up to 4.8% of total formulation in fully cooked meats and poultry products is allowed. The antimicrobial activity of lactic acid is not solely a result of a lowering of pH but also has been demonstrated to disrupt the outer membrane of gram-negative bacteria *(1)*. A recent application for lactic acid (2%) as an antimicrobial spray applied to animal carcasses to reduce surface populations of *Escherichia coli* O157:H7 and *Salmonella* has been reported *(7)*. Sorbic acid (pK_a = 4.76) and its potassium salt are widely used in foods at a concentration of 0.02 to 0.3% to inhibit yeast and molds but do have antibacterial activity as well *(27)*. EDTA, a metal ion chelator, is used in foods as a disodium salt and a disodium-calcium complex at levels of 50 to 150 ppm *(25)*. The inhibition of bacteria by EDTA is presumed to be due to chelation of divalent cations found in the cell

TABLE 3. *Population of* Salmonella *on fresh-cut cantaloupe prepared from treated inoculated whole melons after storage at 5°C for up to 7 days*

	Population[b]					
	Day 0		Day 3		Day 7	
Treatments[a]	Log CFU/g	Enrichment	Log CFU/g	Enrichment	Log CFU/g	Enrichment
Control	1.96 ± 0.13		2.31 ± 0.10		2.66 ± 0.13	
Nisin-EDTA	ND	+	ND	+	0.48 ± 0.11	+
Nisin-NaL	ND	+	ND	+	0.35 ± 0.10	+
Nisin-KS	ND	+	ND	+	0.51 ± 0.12	+
NaL-KS	ND	+	ND	+	0.23 ± 0.13	+
Nisin-NaL-KS	ND	+	ND	+	ND	+

[a] Concentrations of the antimicrobial agents were 50 μg/ml (nisin), 0.02 M (EDTA), 2% (NaL), and 0.02% (KS).

[b] Preparation of fresh-cut pieces was performed within 30 min of washing treatments. Values are means ± standard deviations of three trials with duplicate determinations per trial. ND, none detected by direct plating (limit of detection was 2 CFU/g). +, positive in 100 g by enrichment.

TABLE 4. *Survival of* Salmonella *on directly inoculated fresh-cut pieces after washing treatments and storage at 5°C for up to 7 days*

Treatments[a]	Population (log CFU/g)[b]		
	Day 0	Day 3	Day 7
Control	3.42 ± 0.13 A	3.91 ± 0.14 A	4.46 ± 0.11 A
Water	3.02 ± 0.11 A	3.85 ± 0.12 A	4.58 ± 0.10 A
Nisin-EDTA	3.07 ± 0.14 A	3.15 ± 0.10 A	3.18 ± 0.13 A
Nisin-NaL	2.62 ± 0.11 B	2.69 ± 0.14 B	2.58 ± 0.10 B
Nisin-KS	2.82 ± 0.12 B	2.88 ± 0.11 B	2.78 ± 0.10 B
NaL-KS	2.40 ± 0.10 B	2.49 ± 0.11 B	2.52 ± 0.13 B
Nisin-NaL-KS	2.02 ± 0.13 C	2.25 ± 0.12 C	2.18 ± 0.10 C

[a] Concentrations of antimicrobial agents were 50 μg/ml (nisin), 0.02 M (EDTA), 2% (NaL), and 0.02% (KS).

[b] Values are means ± standard deviations of three experiments with duplicate determinations per trial. Means not followed by the same letter in each column are significantly different ($P < 0.05$).

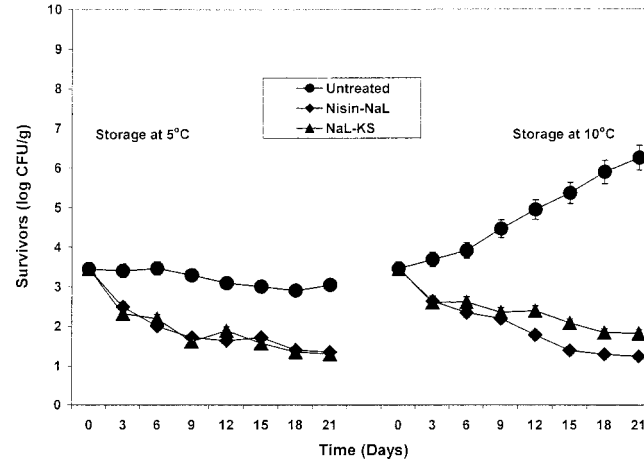


FIGURE 3. *Survival of a five-strain cocktail of* Salmonella *on fresh-cut cantaloupe pieces washed in nisin (50 μg/ml)-NaL (2%) and NaL (2%)-KS (0.02%) and stored at 5 and 10°C.*

wall. In addition to use as a preservative, the salts of EDTA also enhance color retention and inhibit discoloration.

In our in vitro studies conducted in broth media adjusted to an initial pH of 4.0 to 6.8, none of the four compounds when tested individually exhibited significant inhibitory activity against the cocktail of *Salmonella* strains. By contrast, all combinations of the antimicrobial compounds (nisin-NaL, nisin-KS, nisin-EDTA, and NaL-KS) were bactericidal in broth at all three initial pHs (4.0, 5.0, and 6.8) except for nisin-EDTA, which was bacteriostatic at pH 6.8, a pH that approximated the pH of cantaloupe fleshy tissue. The bactericidal activity of nisin (50 μg/ml)–lactate (500 mM) and nisin (50 μg/ml)–EDTA (50 mM) against *Salmonella* Typhimurium suspended in PBS or MOPS buffers adjusted to pH 7.0 after addition of antimicrobial compounds was previously reported *(10).* The strains of *Salmonella* used in our study appear to be more resistant to nisin-EDTA in vitro than *Salmonella* Typhimurium.

Our results for reduction of *Salmonella* when present on the surface of whole cantaloupe were similar to in vitro results in that, when tested individually, all four antimicrobial compounds were ineffective, as were water washes. We previously reported that treatment of cantaloupe melon with water, EDTA (0.02 M), and nisin (10 μg/ml) did not cause significant reductions of native microflora on whole cantaloupe *(31).* However, when tested in combination, significant reductions of *Salmonella* (~3 log CFU/cm^2) were obtained before storage (day 0) of inoculated cantaloupe for nisin-EDTA, nisin-NAL, nisin-KS, NaL-KS, and nisin-NaL-KS. It is interesting to note that nisin-EDTA was not

TABLE 5. *Quality rating of inoculated and treated fresh-cut pieces during storage at 5°C for 7 days*

Treatments[a]	Selected observations[b]	Ratings[c]		
		Appearance	Odor	Overall acceptability
Control 1[d]	Looks good, highly acceptable	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
Control 2 (+*Salmonella*)[e]	Looks good, highly acceptable	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
Control 3[f]	Soft, slightly dark in color	5.0 ± 0.7	5.8 ± 0.8	5.0 ± 0.7
Nisin	Mushy, unacceptable	2.0 ± 0.7	5.2 ± 0.4	3.2 ± 0.4
EDTA	Good, not soft	6.0 ± 0.7	6.8 ± 0.5	6.2 ± 0.5
Nisin-EDTA	Mushy, unacceptable	3.2 ± 0.5	4.2 ± 0.5	3.2 ± 0.5
NaL	Firm, looks good, acceptable	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Nisin-NaL	Firm, looks good, acceptable	9.8 ± 0.4	8.2 ± 0.5	8.8 ± 0.5
KS	Less soggy, unacceptable	4.2 ± 0.5	4.2 ± 0.5	5.0 ± 0.7
Nisin-KS	Soggy, unacceptable	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
NaL-KS	Less firm, looks good, acceptable	7.2 ± 0.5	6.0 ± 0.7	5.8 ± 0.5
Nisin-NaL-KS	Slightly mushy, less acceptable	5.2 ± 0.5	5.4 ± 1.1	4.2 ± 0.5

[a] Concentrations of antimicrobial agents were 50 μg/ml (nisin), 0.02 M (EDTA), 2% (NaL), and 0.02% (KS).

[b] Words used to describe fresh-cut melon and acceptability if offered for consumption.

[c] Rating scale (1 to 10), with 10 being the maximum acceptable score. Scores shown are means ± standard deviations.

[d] Control 1 was fresh-cut melon prepared on the day of evaluation.

[e] Control 2 was fresh-cut melon prepared on the day of evaluation and inoculated with *Salmonella* without washing treatment.

[f] Control 3 was fresh-cut melon 7 days postinoculation stored at 5°C without washing treatment.

effective against the *Salmonella* strains in broth at pH 6.8 but was effective against these bacteria when present on whole cantaloupe. The population reductions were similar to that obtained after treatment of inoculated whole cantaloupe with chlorine (1,000 ppm) or hydrogen peroxide (5%) *(32, 34).* In this study and in our past study *(34),* even though significant population reductions were obtained after storage of the inoculated cantaloupes, the treatment efficacy was reduced. The reduced efficacy of sanitizer treatments after storage of inoculated cantaloupe could be because of strong attachment of the bacteria to the melon surfaces, attachment to sites inaccessible to aqueous sanitizers, or biofilm formation. This phenomenon needs to be taken into account when evaluating sanitizers for reduction of pathogens on produce. As found for chlorine and hydrogen peroxide *(34),* none of the five treatment combinations totally eliminated *Salmonella* from the cantaloupe surface or prevented the transfer of *Salmonella* from the whole cantaloupe to the interior flesh when preparing fresh-cut pieces.

Populations of *Salmonella* directly inoculated to fresh-cut pieces of cantaloupe were also not eliminated by any of the five treatment combinations tested. The treatment combination of nisin-NaL-KS was most effective, followed by NaL-KS, resulting in reductions of 1 to 1.4 log CFU/g. Statistically similar population reductions to that obtained with NaL-KS were found for nisin-NaL and nisin-KS. Treatment with nisin-EDTA was ineffective against *Salmonella* on fresh-cut pieces. This result was unexpected because a previous study in our laboratory demonstrated an approximately 2-log CFU/g reduction in total aerobic plate counts after treatment of fresh-cut pieces with nisin (10 μg/ml)–EDTA (0.02 M) *(31).* It is possible that the reduction seen in the previous experiment was for gram-positive bacteria that are more susceptible to the antimicrobial effects of nisin. Treatment of fresh-cut pieces with nisin-NaL-KS or nisin-KS appears not to be practical, however, because of undesirable effects on quality. Treatment with the combinations NaL-KS or nisin-NaL does appear acceptable from a quality standpoint on the basis of the parameters measured.

After the required regulatory approvals and on the basis of the results of both our previous study *(31)* and this study, treatment of whole cantaloupe with nisin-EDTA and fresh-cut pieces treated with NaL-KS or nisin-NaL could lead to both increased shelf life and a reduced risk of foodborne illness by contamination with *Salmonella.* The use of nisin for treating fresh-cut melon might also reduce the risk of contamination with *Listeria monocytogenes (18).*

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